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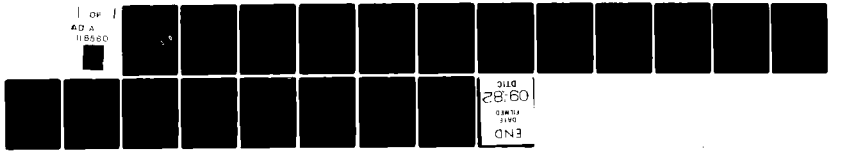

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Fischer 344 rats were treated with 10,20, or 30 mg/kg of nonadecafluorodecanoic acid (NDFDA) on day 12 of gestation. Negative controls were given the vehicle (propylene glycol and water; 50:50) and positive controls were given a single ip injection of 500 mg/kg hydroxyurea (diluent, physiological saline). Developmental eruption, eye opening, and growth) and behavior. NDFDA was found to produce increased visuomotor development (visual placing). It is also caused decreased prenatal and postnatal weight gain, delayed cliff avoidance and surface righting, decreased open field rearing, aberrant swimming

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FINAL REPORT

A NEUROBEHAVIORAL STUDY ON RATS USING A MODEL PERFLUORINATED ACID , NDFDA

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A NEUROBEHAVIORAL STUDY ON RATS USING A MODEL PERFLUORINATED ACID,NDFDA

by

Inez R. Bacon

ABSTRACT

Fischer 344 rats were treated with 10, 20, or 30 mg/kg of nonadecafluorodecanoic acid(NDFDA) on day 12 of gestation. Negative controls were given the vehicle (propylene glycol and water;50:50) and positive controls were given a single ip injection of 500 mg/kg hydroxyurea(diluent,physiological saline). Developmental parameters in the offspring included physical development(pinna detachment,incisor eruption, eye opening, and growth) and behavior. NDFDA was found to produce delayed motor development(forward locomotion with head low and body low) and increased visuomotor development(visual placing). It also caused decreased prenatal and postnatal weight gain,delayed cliff avoidance and surface righting, decreased open field rearing, aberrant swimming development and increased auditory startle. The observed effects indicate that NDFDA is a potential toxic neurochemical teratogen.

Acknowledgement

I am grateful to the Air Force Office Office of Scientific Research for granting the funds to perform this study. My sincere thanks are also extended to Ms. Denise Albury and Mr. Alan Johnson for technical assistance.

INTRODUCTION

Nonadecafluorodecanoic acid($CF_3(CF_2)_8CO_2H$) is a model perfluorinated acid which closely mimics a Fluorad brand flurochemical surfactant which is used by the Air Force in foams of fire extinguishers. In addition, other related perfluoro acid derivatives arewidely used by industry and the general public. For example, the wide variety of applications and uses made possible through the unique properties of the perfluoro acid derivatives include: paints,polishes, metal cleaners,coatings,dying agents, inks, soaps and detergents, insecticides and herbicides and numerous other agents which may be potentially hazardous to the Air Force and the public's health.

The available literature on the toxicity of NDFDA is limited to an in-house study at the Aerospace Medical Research Laboratory,Toxic Hazard Division, Wright-Patterson AFB,OH. NDFDA is cited as causing histopathological damage to the thymus, bone marrow,stomach,mesentery,liver, and testes in male rats (Andersen,1979). These results show the effect that exposure to NDFDA may have on men, but do not depict the potential hazard to women.As a result of this deficit,another study was conducted to determine the teratogenic and/or embryotoxic potential of NDFDA. In this study,evidence of teratogenicity was not observed in fetuses of dams administered NDFDA by gavage on day 9 of gestation. Toxic effects in fetuses occurred only in the presence of dose-related maternal toxicity(less maternal body weight gain at 30 mg/kg/day 12 of gestation). There was a significant decrease in the mean fetal body weight among litters of rats treated with 30 mg/kg/day 12 of gestation. There was also an increase in resorptions and a corresponding decrease in the number of live fetuses per litter.There was no significant difference between control and experimental soft tissue or skeletal anomalies at any dosage level(Bacon et al, 1980). Butcher et al(1972) report that some compounds, when administered at the critical stages of organogenesis in doses which do not cause malformations, can induce postnatal toxic effects and lasting behavioral changes in the offspring. This hypothesis is also supported by Tanimura(1976) and Frohberg(1975;1977). Since fetal toxicity paralled maternal toxicity,the present study was conducted to determine the effect of NDFDA on the central nervous system of developing pups.

MATERIALS AND METHODS

Materials and animals

NDFDA(purity 98%) was used in this study. Fischer 344 rats were housed in polypropolene cages in a room with controlled temperature(70-76°F)and light cycle(12 h light and 12 h dark). The animalswere maintained on Ralston Purina Laboratory Chow and tap water ad libitum. Rats were allowed to acclimatize one week prior to treatment . The day on which sperm was observed in a vaginal smear was considered day 0 of gestation.

Procedure

The procedure used was that of Vorhees et al(with a few modifications,1979). NDFDA was given by gavage in propylene glycol and water(50:50) at a dose volume of 2 ml/kg body weight. Pregnant rats were given 10, 20, or 30 mg/kg NDFDA on day 12 of gestation. Negative controls were given the vehicle and positive controls were given a single ip injection of 500 mg/kg of hydroxyurea(HU)(diluent,physiological saline) on day 12 of gestation.Dose levels were selected based on data from the previous teratogenic study.

Due tothe high mortality rate of the offspring,litters with fewer than 8 offspring were not discarded. All offspring in each litter were used in the experiment. All rats were weighed at weekly intervals throughout the experiment except during breeding. All newborns were sexed, weighed , and marked with non-toxic colored markers.After weaning,the pups were marked by clipping the ears.

Pinna detachment was observed daily from day 1 until both pinna were detached. Incisor eruption was observe daily from day 1 until both upper and lower incisors had erupted in all test pups.Eye opening was observed daily from day 10 until both eyes of all test pups were opened(Irwin,1968).Forward locomotion was observed daily from day 1 until each of the following aspects of forward locomotion had appeared in all test pups: first day all pups showed forward locomotion with bodies carried low , first day all pups lift their bodies above surface during forward locomotion but with heads remaining down, and first day all pups showed forward locomotion with both their bodies and heads lifted(Altman and Sudarshan,1974).

Surface righting,cliff advoidance, and auditory startle were performed using the methods of Brunner et al(1978).The only modification was use of a bicycle horn rather than an automobile horn for the stimulus in the test for auditory startle.

Swimming development was tested using the methods of Vorhees et al(1979). However, one modification was made. This modification was the use of 4'X2' glass pool(built by the author) instead of a tank. Visual placing was performed using the methods of Marshall and Teitelbaum(1974).

Open field observations were made on day 21 for 4 consecutive days for 3 min/day and scored for the total number of sections entered, total number of rearing instances, total number of fecal boluses deposited, and latency to begin exploration during the 3 min daily test period. The open field was a 4'X4' wooden box. The floor was black with 9 circles.Each circle was divided in halves(total of 18 sections). The floor was covered with clear plexiglass to facilitate cleaning after each pup. This open field box was also built by the author.

The data was analyzed using either the t-test or Mann-Whitney rank sum test.

RESULTS

Only one mother in the 30 mg/kg group died during the period of gestation(day 20). An autopsy of this mother revealed a juandiced liver,white patches on the kidneys, and 11 resorptions. A total of 9 mothers(one 10 mg/kg,six 20 mg/kg,two 30 mg/kg) were sacrificed to determine pregnancy. All females in the 20 mg/kg group revealed early and/or late resorptions, and white patches on the kidneys and liver. Two females in the 20 mg/kg group displayed an abundance of clear fluid in the abdominal cavity. One female in the 30 mg/kg group revealed a jaundiced liver and 12 late resorptions, and one female had a hard bladder and 4 late resorptions. All post-partums displayed increased defecation.

There was no significant difference between the maternal body weight gain of negative and positive controls and the 10 mg/kg group(Table 1). However, body weight gain was highly significant in the 20 mg/kg($p \ll 0.001$) and the 30 mg/kg groups($p < 0.02$) as compared to the negative controls. In addition, all postpartums in the 30 mg/kg group continued to lose weight and most died within 30 to 40 days.

The number of live pups per litter did not show any statistically significant difference(Table 2). Only one pup in the positive control group was stillborn. A significant number of pups died by day 4 and day 7 in the 30 mg/kg group($p < 0.05$). The death rate was highly significant by day 14 and 21($p \ll 0.005$). A significant amount of weight loss was observed in all pups which died in the 20 mg/kg and 30 mg/kg groups. The survival rate of male and female offspring are summarized

in Table 3. There was no significant difference between negative control groups and the other groups, with the exception of the positive control and the 30 mg/kg, by day 7 and day 21. Both males and females showed a significantly higher death rate than males and females of the other groups. The death rate of intragroup males and females were significant only in the 30 mg/kg group($p < 0.05$) and only by the 7th day of life. However, the survival rate of males were greater than females in all groups. The negative control displayed 2 cases of curled tail and the positive control displayed 15 kinked tails, 11 microopthalmia, and 1 notched tail. There were 12, 6, and 5 cases of notched tail in the 10, 20, and 30 mg/kg groups, respectively. However, notched tails were not discernable until 6 to 10 days of postnatal life.

The body weight gain of the offspring after birth are summarized in Table 4. Both male and female offspring showed a significant decrease in weight with the exception of the 10 mg/kg group(females). Retarded growth continued in both male and female positive controls and the 10 mg/kg females on day 7. Only the the 20 mg/kg males showed significant growth retardation on day 14. By day 21, no significant differences in body weight gain were observed in any groups with the exception of males in the 30 mg/kg group. There was an increase in body weight gain of these males. This apparent increase could possibly be due to the high mortality rate of pups in that group.

Pinna detachment and eye opening were not significantly different in any groups(Table 5). With the exception of the 10 mg/kg group, there was no significant difference of upper and lower incisor eruptions. The 20 mg/kg group showed a significant delay in cliff avoidance, surface righting, and auditory startle.

The first day all pups showed forward locomotion with head and body low did not differ significantly in the negative control, positive control, and 10 mg/kg groups(Table 6). Forward locomotion with head low and body low were significantly delayed in the 20 mg/kg group($p < 0.05$) and highly significant in the 30 mg/kg group($p << 0.001$). The only group which showed a significant delay in forward locomotion with head low and body up were the positive controls and the 30 mg/kg group. There was no significant difference in forward locomotion with head up and body up for any group. The only group which showed a significant difference of visual placing was the 30 mg/kg group. Visual placing was acheived approximately 2 days sooner than the negative controls, positive controls, and the 10 mg/kg group. It was achieved approximately one day sooner than the 20 mg/kg group.

In open field testing,males in the 10 mg/kg group showed a significant decrease in the number of sections entered than males of other groups(Table 7). Females in the 30 mg/kg group showed a significant increase in the number of sections entered than females in other groups. One female in the 20 mg/kg group walked with an irregular gait(front feet crossed each other and stepped high). The number of rearings in the 20 mg/kg and 30 mg/kg group of males were significantly lower than males of the positive control,negative control, and 10 mg/kg groups. With the exception of the positive control females(increased latency to start), no statistically significant differences among the groups were observed in other variables.

Only females in the positive control group showed a significant delay in swimming direction(Table 8). Both males amd females of the 10 mg/kg group and females in the positive control group showed a significant delay in swimming angle. Males of the 10 mg/kg group also showed a significant delay in forelimb inhibition. However, males in the 30 mg/kg group showed advanced forelimb inhibition as compared to the negative controls.

DISCUSSION

Toxicity of NDFDA to mothers in the 20 mg/kg and 30 mg/kg groups was manifested by maternal death, decreased body weight gain,changes in the liver, kidneys , and bladder. Developmental toxicity of NDFDA to offspring was expressed through resorptions, intrauterine and postnatal growth retardation, notched tail, and decreased postnatal survivors . Other markers were delayed reflex development (e.g. surface righting,cliff avoidance,and auditory startle), motor and visuomotor development, and neuromuscular ability. The delayed reresponse of the 20 mg/kg and 30 mg/kg groups to forward locomotion indicated decreased motor development. Since all groups acheived forward locomotion with body up and head up at approximately the same time, the previous delays appear to be transitory. NDFDA appear to cause advanced visual placing in the high dose (30 mg/kg). Even though the 20 mg/kg group did not show significant advancement, pups in this group acheived criterion approximately one day sooner than the controls.

Evidence of functional impairment was observed in males in the 10,20, and 30 mg/kg groups. These males showed decreased exploratory behavior(10mg/kg),reduced open field rearing(20mg/kg), and aberrant swimming(10mg/kg,30mg/kg). Females

showed increased exploratory behavior(30mg/kg) and less aberrant swimming. Later testing of these parameters could possibly determine whether this behavior had a transient or permanent effect. In the determination of permanent effects on behavior, one approach has been to use a test-retest procedure(Ordy et al.,1966; Ulleland,1972). Another method involves testing on a variety of tasks over a prolonged period of time(Fink et al.,1967; Borgen et al.,1973;Kletzkin et al.,1964; Werboffet et al.,1961;Werboffet and Havlena,1962;Marai,1963). However, such testing at a later age would give different results.For example, defecation,urination, and exploration in open field vary with age(Michealson et al.,1975). Although behavioral deficits which persist into adulthood appear to be the most serious,delays in the development of certain kinds of behavior could be more sensitive indicators of behavioral teratogenic effects than tests of adult behavior(Frey,1971).

Behavioral changes induced by prenatal administration of NDFDA have not been previously reported.Thus, comparision of this study with others using this chemical is not possible.However, much could be learned about NDFDA if histological studies of the brain were performed. In addition, F_2 litters should be examined to determine prenatal and postnatal behavioral aberrations.

Table 1. Maternal Weight gain(in grams) durning pregnancy and resorptions per nonparturition mothers +

Treatment	Number of pregnant animals	Day 0	Day 21*	Number of nonparturients	Number of resorptions/nonparturients
Negative control (vehicle)	10	198±15.4	262.7±24.5	0	0
Positive control 500 mg/kg (Hydroxyurea)	10	185.9±10.3	243.9±25.2	0	0
10 mg/kg NDFDA	10	200.4±18.4	263.6±25.4	1	14
20 mg/kg NDFDA	10	198.4±20.3	185.9±32.1[a]	6	6.3±3.4[c]
30 mg/kg NDFDA	10	198.9±20.9	212.7±50.7[b]	3	9.0±4.4

+Mean ± S.D.

*Weight prior to parturition.

[a]Significantly different from negative control, $p << 0.001$.

[b]Significantly different from negative control, $p < 0.02$.

[c]Significantly different from negative control, $p < 0.01$.

Table 2. Effects on the offspring before weaning of nonadecafluorodecanoic acid and/or hydroxyurea treated mothers

Treatment	No. of mothers	At birth		Postnatal survival			
		No. of live(+)	No. of dead(+)	Day 4(+)	Day 7(+)	Day 14(+)	Day 21(+)
Vehicle	10	99 (9.9 ± 1.4)	0	96 (9.6 ± 1.6)	96 (9.6 ± 1.6)	93 (9.3 ± 1.8)	92 (9.2 ± 1.8)
500 mg/kg HU	10	107 (9.9 ± 1.6)	(0.1 ± 0.3)	103 (9.5 ± 2.4)	99 (9.1 ± 3.5)	91 (8.4 ± 4.4)	91 (8.4 ± 4.4)
10 mg/kg NDFDA	9	82 (9.1 ± 1.5)	0	82 (9.1 ± 1.5)	82 (9.1 ± 1.5)	81 (9.0 ± 1.6)	80 (8.9 ± 1.7)
20 mg/kg NDFDA	4	40 (10.0 ± 1.4)	0	35 (8.8 ± 3.4)	32 (8.5 ± 3.3)	31 (8.0 ± 2.7)	29 (6.8 ± 2.6)
30 mg/kg NDFDA	7	56 (8.0 ± 3.3)	0	38 (5.4 ± 5.3)[a]	37 (5.3 ± 5.2)[a]	30 (4.7 ± 4.9)[b]	23 (4.4 ± 4.1)

(+) Mean ± S.D.

[a] Significantly different from negative control , $p < 0.05$.

[b] Significantly different from negative control , $p << 0.005$.

Table 3. Survival rate of male and female offspring of rats treated with NDFDA and / or Hydroxyurea

Treatment	Sex	Age in days (Number of dead) 1	7	14	21	Total dead
Negative control	M	41	40(1)	39(2)	39(0)	2
	F	58	56(2)	54(2)	53(1)	5
Positive control (HU)	M	54	50(4)	46(4)	46(0)	8
	F	53	49(4)	45(4)	45(0)	8
10 mg/kg NDFDA	M	42	42(0)	42(0)	42(0)	0
	F	40	40(0)	39(1)	38(1)	2
20 mg/kg NDFDA	M	22	19(3)	18(1)	18(0)	4
	F	18	14(4)	14(0)	11(3)	7
30 mg/kg NDFDA	M	36	28(8)[a]	28(0)	16(12)[a]	20
	F	20	10(10)[a]	9(1)	7(2)[a]	13

[a] Significantly different from negative control , $p \ll 0.001$.

Table 4. Preweaning offspring growth in rats treated with NDFDA and /or Hydroxyurea [a]

Sex	Treatment	Age (days) 1	7	14	21	n[b]
Males	Negative control	5.3 ± 0.3	9.6 ± 1.1	16.8 ± 2.1	23.4 ± 3.4	10/10
	Positive control	4.5 ± 0.5	8.3 ± 1.2[e]	15.5 ± 2.0	22.2 ± 3.3	10/9
	10 mg/kg NDFDA	4.9 ± 0.5[d]	8.8 ± 1.2	15.8 ± 2.1	22.4 ± 2.5	9/9
	20 mg/kg NDFDA	4.5 ± 0.3[c]	8.3 ± 1.1	14.2 ± 3.5	25.7 ± 1.8	4/4
	30 mg/kg NDFDA	4.3 ± 1.1[e]	8.6 ± 0.7	17.1 ± 3.0	27.7 ± 3.9[d]	7/2
Females	Negative control	5.1 ± 0.3	9.5 ± 1.2	16.4 ± 2.5	22.5 ± 5.1	10/10
	Positive control	4.2 ± 0.6[c]	8.1 ± 1.1[d]	14.8 ± 1.6	21.7 ± 2.3	10/9
	10 mg/kg NDFDA	4.7 ± 0.5	8.1 ± 1.1[d]	15.1 ± 2.3	21.7 ± 2.7	9/9
	20 mg/kg NDFDA	4.3 ± 0.3[c]	7.2 ± 1.0	13.2 ± 2.6	21.9 ± 4.3	4/4
	30 mg/kg NDFDA	3.6 ± 1.3[e]	8.4 + 1.0	16.8 ± 1.3	23.6 ± 0.9	7/2

[a] Weight in grams expressed as means ± S.D.

[b] Number of litters/group on day 21.

[c] Significantly different from negative, $p \ll 0.001$.

[d] Significantly different from negative control, $p < 0.05$.

[e] Significantly different from negative control, $p < 0.01$.

Table 5. Physical and reflex development of offspring from rats treated with NDFDA and / or Hydroxyurea

Treatment	Early physical development			Early reflex development			n[a]
	Pinna detachment	Upper and lower incisor eruption	Eye opening	Cliff avoidance	Surface righting	Audiotory startle	
Negative control	3.0 ± 0	11.1 ± 1.0	18.6 ± 0.6	8.9 ± 1.7	7.2 ± 1.5	13.1 ± 0.8	10
Positive control	3.0 ± 0	11.8 ± 0.8	18.3 ± 0.7	9.3 ± 1.5	8.2 ± 2.7	13.0 ± 1.2	10
10 mg/kg NDFDA	3.0 ± 0	12.1 ± 0.6[b]	18.8 ± 0.7	9.7 ± 1.5	7.7 ± 1.1	13.2 ± 0.7	9
20 mg/kg NDFDA	3.0 ± 0	11.6 ± 0.5	19.0 ± 0	10.5 ± 1.3	9.7 ± 2.1[b]	13.5 ± 0.6	4
30 mg/kg NDFDA	3.0 ± 0	10.1 ± 0.2	18.0 ± 0	11.3 ± 1.0[c]	9.6 ± 1.4[b]	12.1 ± 0.2	4

* Age(in days) at which pups reached criterion.

[a] Number of litters at time of testing.

[b] Significantly different from negative control , $p < 0.05$.

[c] Significantly different from negative control , $p < 0.02$.

Table 6. Motor and visuomotor development of offspring of rats treated with NDFDA and / or Hydroxyurea

Treatment	Mean(±S.D.)day all showed forward locomotion with: Head and body low	Head low and body up	Head and body up	Mean(±S.D.)day all showed: Visual placing	Number of litters
Negative control	5.4 ± 1.9	8.8 ±0.6	18.6 ± 0.6	21.3 ± 1.0	10
Positive control	5.4 ± 0.7	11.2 ± 2.2[a]	18.3 ± 0.7	21.9 ± 1.0	9
10 mg/kg NDFDA	6.2 ± 1.6	8.8 ± 2.1	18.8 ± 0.7	21.5 ± 0.9	9
20 mg/kg NDFDA	7.7 ± 0.7[a]	11.5 ± 2.1	19.0 ± 0	20.6 ± 1.0	4
30 mg/kg NDFDA	11.1 ± 1.2[b]	13.2 ± 0.6[c]	18.0 ± 0	19.8 ± 0.6[d]	4

[a] Significantly different from negative control, $p < 0.05$.

[b] Significantly different from negative control, $p << 0.001$.

[c] Significantly different from negative control, $p << 0.005$.

[d] Significantly different from negative control, $p < 0.02$.

Table 7. Mean(± S.D.) of open field-behavior for offspring of NDFDA and / or Hydroxyurea treated rats

Treatment	Sex	No. of pups	Mean score of 4 days of testing at 3 min / day: Number of Sections entered	Rearings	Fecal boluses	Urination	Latency to start(sec)
Negative control	Males	39	4.1 ± 3.1	19.0 ± 6.5	0.2 ± 0.5	0.3 ± 0.3	28.7 ± 13.3
Positive control (500 mg/kg HU)		46	6.8 ± 6.0	26.3 ± 9.7	0.2 ± 0.3	0.5 ± 0.5	34.6 ± 11.1
10 mg/kg NDFDA		39	1.8 ± 3.2[a]	21.1 ± 8.5	0.2 ± 0.4	0.3 ± 0.5	34.2 ± 11.6
20 mg/kg NDFDA		22	2.9 ± 1.9	8.3 ± 5.9[b]	0.2 ± 0.7	0	23.2 ± 4.2
30 mg/kg NDFDA		19	5.9 ± 2.7	8.1 ± 4.0[a]	0	0.2 ± 0.3	38.5 ± 8.5
Negative control	Females	56	2.4 ± 1.9	16.6 ± 8.2	0.2 ± 0.3	0.2 ± 0.4	28.6 ± 13.1
Positive control (500 mg/kg HU)		45	4.2 ± 2.5	22.8 ± 7.6	0.1 ± 0.2	0.5 ± 0.5	43.4 ± 12.4[b]
10 mg/kg NDFDA		38	5.1 ± 4.8	24.9 ± 10.0	0.2 ± 0.3	0.1 ± 0.1	30.5 ± 9.2
20 mg/kg NDFDA		11	2.1 ± 2.4	12.6 ± 11.9	0.2 ± 0.4	0	17.6 ± 7.0
30 mg/kg NDFDA		9	6.7 ± 2.3[a]	7.3 ± 3.8	0	0.1 ± 0.2	37.1 ± 9.6

[a] Significantly different from negative control, $p \leq 0.01$.

[b] Significantly different from negative control, $p \leq 0.02$.

Table 8. Swimming development of offspring of rats treated with NDFDA and/or Hydroxyurea

		Mean day(± S.D.) all reached criterion			
Treatment	Sex	Swimming direction	Swimming angle	Forelimb inhibition	Number of pups *
Negative control	M	12.7 ± 2.9	17.0 ± 2.4	23.1 ± 2.6	39
	F	13.3 ± 3.2	17.1 ± 2.9	23.2 ± 5.3	53
Positive control (500 mg/kg HU)	M	13.7 ± 2.5	17.1 ± 2.0	24.1 ± 3.5	46
	F	12.5 ± 2.3[a]	16.0 ± 1.9[a]	22.4 ± 3.0	45
10 mg/kg NDFDA	M	12.7 ± 1.9	15.5 ± 2.2[c]	24.0 ± 2.9[a]	39
	F	13.3 ± 2.0	15.8 ± 2.0[b]	23.7 ± 2.9	38
20 mg/kg NDFDA	M	12.9 ± 0.9	17.5 ± 2.2	23.6 ± 2.3	18
	F	13.2 ± 1.3	17.9 ± 2.5	23.3 ± 2.6	11
30 mg/kg NDFDA	M	12.9 ± 1.1	17.1 ± 1.7	20.5 ± 1.7[d]	16
	F	12.7 ± 0.8	17.4 ± 1.5	22.3 ± 2.6	7

* Represents the number of pups at the end of testing.

[a] Significantly different from the negative control, $p < 0.05$.

[b] Significantly different from negative control, $p < 0.02$.

[c] Significantly different from negative control, $p << 0.001$.

[d] Significantly different from negative control, $p < 0.01$.

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